Plant Physiol. (1973) 51, 1089–1094

The Role of Phytochrome in an Interaction with Ethylene and Carbon Dioxide in Overcoming Lettuce Seed Thermodormancy[1]

Received for publication November 10, 1972

FAYEK B. NEGM, O. E. SMITH, AND JUNJI KUMAMOTO
Department of Plant Sciences, University of California, Riverside, California 92502

ABSTRACT

Ethylene and CO_2 were used to control induction of germination in thermodormant lettuce seed (*Lactuca sativa* L.). These experiments ultimately showed that germination depends on the presence of an active form of the phytochrome. The phytochrome system is functional and stable at 35 C, a temperature which completely inhibits germination. Phytochrome responses to red or far red light and darkness showed that this inhibition of germination under light must be due to some other block(s) rather than to a direct inactivation of the phytochrome system itself. A postred radiation increase in lettuce seed germination that is not reversed by far red light was observed. The CO_2 requirement for C_2H_4 action is not due to a change in the medium's pH; addition of C_2H_4 plus CO_2 at the start of imbibition did not result in as much germination as when they were added several hours after imbibition. This reduction in germination, when the gases are added at the start of imbibiton, is due to CO_2.

Some varieties of lettuce (*Lactuca sativa* L.) seed, such as Great Lakes, have been considered light-insensitive because they germinate equally well in light or darkness at 20 to 25 C; however, at 35 C their germination is completely inhibited under either condition (5). In a previous report (27), we demonstrated that a combination of C_2H_4 with CO_2 will completely overcome lettuce seed thermodormancy at 35 C under continuous light. This combination was effective, if it was added to seeds either at the start or after several days of imbibition.

This report objective is to give more details on the effects of various concentrations of C_2H_4 and CO_2 on thermodormant lettuce seed germination and also to show that, even at this high temperature, their action is dependent on a type of phytochrome light response.

MATERIALS AND METHODS

Fifty lettuce seeds (*Lactuca sativa* L. var. Mesa 659) were imbibed in a 50-ml Erlenmeyer flask on two layers of Whatman No. 1 filter paper (4.25 cm in diameter) moistened with 2 ml of glass-redistilled water. After the flasks containing laboratory air were sealed with rubber serum caps, C_2H_4 or C_2H_4 plus CO_2 were injected to the desired concentrations. Both the addition of C_2H_4 and CO_2 and analysis of the actual concentration of the gases in each flask were carried out as previously reported (27). Gases were injected at the start of imbibition or after several hours of imbibition under air as indicated below. Germination was carried out under continuous cool white light (80 ft-c), in continuous darkness, or in darkness after a short illumination of combinations of red and far-red light 4 hr after the start of imbibition or as otherwise indicated. All experiments and all manipulations (addition of gases, R[2] and FR treatments, and purging with air) were carried out with the main body of all flasks submerged in a 35 ± 0.1 C constant temperature water bath. At this temperature in the absence of C_2H_4 and CO_2, the control seeds used in this study never germinated under any light conditions. In experiments performed in complete darkness or in darkness following either R or FR light treatments, the addition of gases and purging with air were carried out in complete darkness. Each experiment was carried out in quadruplicate and repeated at least four times. Unless otherwise indicated, the germination (protrusion of the radicle) percentage was determined 48 hr after exposure to the gases.

The R light source consisted of four Sylvania F20T12 Gro-Lux fluorescent lamps underlaid with a one-eighth inch thickness of Rohm and Haas 2444 red Plexiglas supported on three-eighths inch clear Plexiglas. The filter was located 30 cm from the top of the flasks; light intensity at this position was approximately 5.3×10^3 erg cm^{-2} sec^{-1}. The FR light source consisted of five GE 150-w weatherproof clear projector floodlights filtered through 7.5 cm of H_2O and one-eighth inch of an FRF 700 filter (Westlake Plastics Co.), also supported on three-eighths inch of clear Plexiglas. The filter was placed 35 cm from the top of the flasks. Irradiation was maintained for 5 min at an intensity of approximately 5.2×10^4 erg cm^{-2} sec^{-1}. The actual light intensity received by the seeds was approximately one-half the reported value because of the presence of serum caps during illumination. The total energy was measured with a YSI Model 65 radiometer, and the spectral distribution was tested with an ISCO spectroradiometer.

RESULTS

Effects of Various Concentrations of C_2H_4 and CO_2 on Lettuce Seed Germination. This experiment was carried out under continuous white light, and C_2H_4 and CO_2 were added at the start of imbibition. Figure 1 clearly indicates that increasing

[1] This work was supported in part by a grant from the California-Arizona Lettuce Seed Research Program.

[2] Abbreviations: R: red; FR: far red.

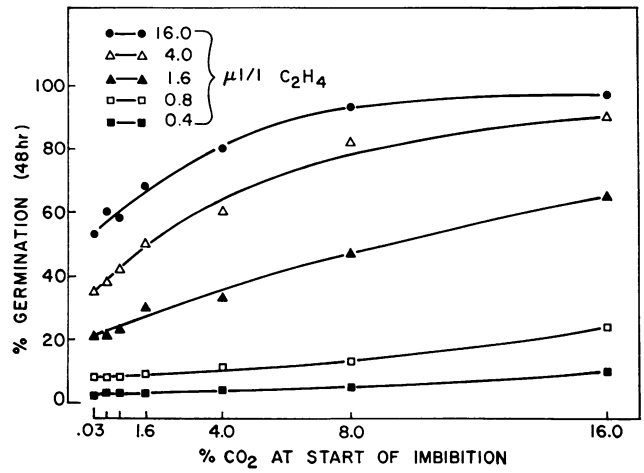

FIG. 1. Effect of varying CO_2 concentration, at selected levels of C_2H_4, on lettuce seed germination at 35 C. Both gases were added at the start of imbibition and the experiments were performed under continuous cool white light.

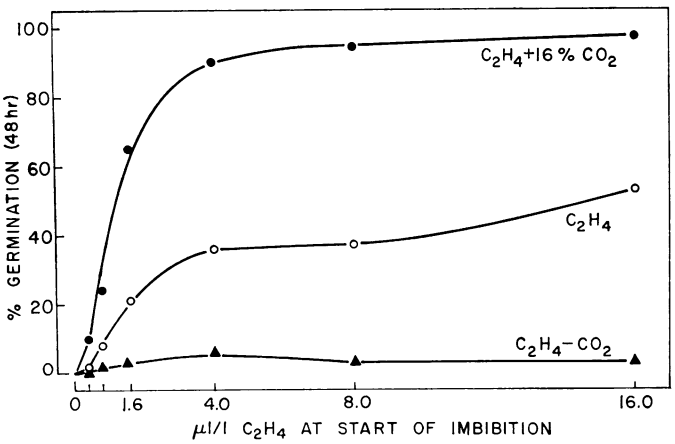

FIG. 2. Effect of different levels of C_2H_4 in the presence of 16% CO_2 or in its absence (20% KOH) on lettuce seed germination at 35 C. Gases were added at the start of imbibition and experiments were conducted under continuous cool white light. Middle curve (C_2H_4) is C_2H_4 in the presence of endogenously produced CO_2 by the seeds.

CO_2 concentration stimulated germination very slightly until C_2H_4 concentration exceeded 0.8 μl/l. There is no indication that CO_2 is inhibitory for C_2H_4 action over the range of concentrations used; yet Figure 2 shows that the absence of CO_2 does prevent germination. These results further confirm our previous report (27) that CO_2 is required for C_2H_4 action in overcoming thermodormancy in lettuce seeds.

Lag Period. The same report (27; see Fig. 1) presented some evidence indicating that imbibed (3 days or more) dormant lettuce seeds will not respond to added C_2H_4 during the first 24 hr after exposure to the gas. However, if these flasks were kept closed and the endogenously produced CO_2 allowed to build up, the seeds eventually germinated. This delay was attributed to the low rate of CO_2 production by the seeds during this period. In the experiment reported here, lettuce seeds were imbibed under continuous white light at 35 C for various periods before C_2H_4 was injected. Flasks were purged with air every 24 hr or before exposure to C_2H_4; germination counts were made 24 hr after exposure to C_2H_4. As can be seen in Figure 3A, lettuce seeds did not respond to C_2H_4 if they were kept imbibed 24 hr or longer before C_2H_4 was given. Figure 3B, however, shows the stimulative action of additional CO_2 in the presence of C_2H_4. In this experiment all the seeds were imbibed for 48 hr before C_2H_4 and various concentrations of CO_2 were injected; germination counts were made 24 hr after the addition of gases. Full germination was obtained when C_2H_4 (16 μl/l) and 8 or 16% CO_2 were used. These data and those reported earlier (27) firmly establish that CO_2 is required for stimulation of thermodormant lettuce seed germination.

Time in Contact with Lettuce Seeds. It was of interest to determine the length of time that C_2H_4 or C_2H_4 plus CO_2 needed to be in contact with lettuce seeds in order to induce germination. In this experiment, seeds were exposed for various lengths of time to gases added initially. Then the flasks were opened, and the seeds were transferred carefully to new flasks containing only air without any gases for the remainder of the imbibition period. The results of this experiment, which was performed under continuous light, indicate that the gases are required for at least 32 to 40 hr to induce maximum germination (Fig. 4).

Effect of pH. The possibility that the CO_2 requirement for C_2H_4 action is due to a lowering of pH was examined, but Table I indicates that this is not so. The use of bicarbonate, citric acid, or sulfuric acid was not effective in replacing the stimulative action of additional CO_2. Sulfuric acid at pH 2.0 was also tested and, while CO_2 (in the presence of C_2H_4) was effective in inducing germination, the radicles were thin and brown in color.

Action of Light on Lettuce Seed Germination at 35 C. It is well known that lettuce seeds become dormant at 35 C, with or without light. In our previous paper (27) and in the first section of this report, all experiments were performed under continuous cool white light. The length of the light period required to induce maximum germination was studied. Seeds were imbibed under laboratory air at 35 C, treated with added C_2H_4 or added C_2H_4 plus CO_2 at the start of imbibition, kept under continuous light for various lengths of time, and then transferred to the dark for the rest of the imbibition period (Fig. 5). The total length of the imbibition period was 48 hr. We found that germination of lettuce seeds in response to C_2H_4 or C_2H_4 plus CO_2 is drastically reduced in complete darkness. For example, C_2H_4 plus CO_2 induced 95% germination in

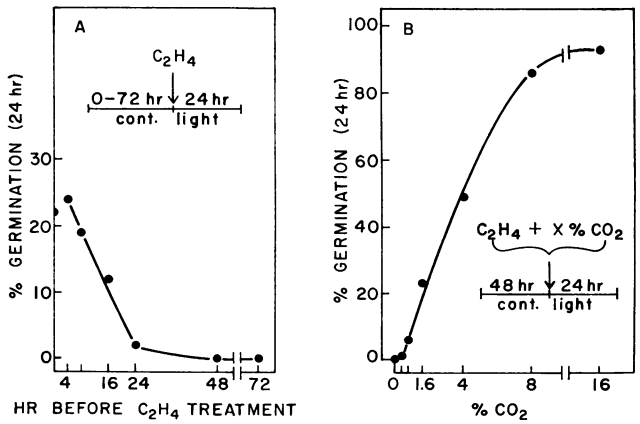

FIG. 3. Response of imbibed lettuce seeds to 16 μl/l C_2H_4 (A) or C_2H_4 plus additional CO_2 (B) at 35 C. A: Seeds were imbibed under air for the indicated time intervals before C_2H_4 was injected. Flasks were purged with air either every 24 hr or before exposure to C_2H_4. B: Seeds were imbibed under air for 48 hr before C_2H_4 and different concentrations of CO_2 were injected. All experiments were performed under continuous cool white light and germination counts were made 24 hr after the addition of gases.

light and 14% in darkness, while C_2H_4, in the presence of endogenously produced CO_2, induced 64% in light and 5% in dark. To determine if C_2H_4 and CO_2 are required during the light exposure, seeds were allowed to imbibe under air and in light for 4 hr, then flasks were transferred to dark, purged with air, and exposed to C_2H_4 plus CO_2 for 44 hr. This treatment resulted (Fig. 6a) in 81% germination; but if C_2H_4 plus CO_2 were given at the start of imbibition, and the seeds kept in the light for 4 hr, then transferred to dark for an additional 44 hr, a total of 55% germination was obtained (Fig. 6b). These results showed that the presence of C_2H_4 plus CO_2 during the first 4 hr of imbibition will reduce germination, and that a shorter light period is sufficient for the induction of germination.

Since this short term light response suggested a phytochrome type system, experiments with R and FR light sources were conducted and all the other manipulations were performed in complete darkness. Seeds were imbibed under air for 4 hr in the dark, purged with air, exposed to R from 1 to 60 min, and then treated with C_2H_4 plus CO_2 for 48 hr in the dark. The results showed that 1 to 30 min of R resulted in 76% germination, which was equivalent to the germination after 1 hr of

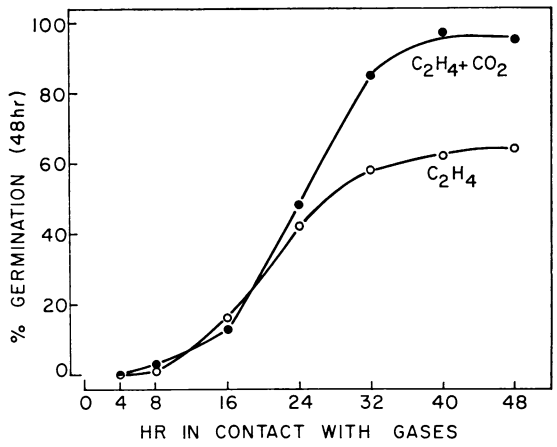

FIG. 4. Effect of the duration of contact with gases on lettuce seed germination at 35 C. Seeds were imbibed under continuous cool white light in the presence of C_2H_4 (16 μl/l) or C_2H_4 plus CO_2 (16%). After each selected imbibition period seeds were transferred to new flasks containing only air for the remainder of the imbibition period.

Table I. *Effect of C_2H_4 and CO_2 (16%) on Lettuce Seed Germination at 35 C*

Seeds were imbibed in 2 ml of various solutions and exposed to the gases at the start of imbibition. All experiments were performed under continuous cool white light.

Treatment[1]	pH	Germination after 48 Hr	
		C_2H_4 (16 μl/l)	C_2H_4 (16 μl/l) + CO_2
		%	
Water		59	95
H_2SO_4 1 mM	2.9	45	98
Citric acid 1 mM	3.1	52	98
Phosphate buffer 10 mM	7.4	27	94
Phosphate buffer 1 mM	7.4	60	96
$NaHCO_3$ 10 mM	8.4	25	95
$NaHCO_3$ 1 mM	7.6	49	98

[1] In the absence of gases, no germination was observed in any of these treatments.

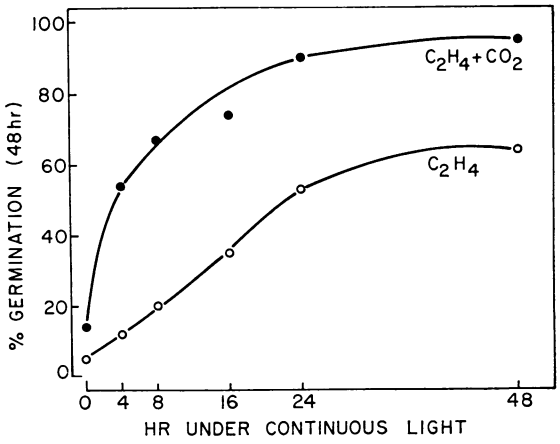

FIG. 5. Response of lettuce seeds treated with C_2H_4 (16 μl/l) or C_2H_4 plus CO_2 (16%) to light. Seeds were treated with gases at the start of imbibition. Flasks were then placed in complete darkness, under continuous cool white light, or exposed to light for the indicated intervals and then transferred to darkness for the remainder of the imbibition period. Germination counts were made 48 hr after the start of imbibition and exposure to gases.

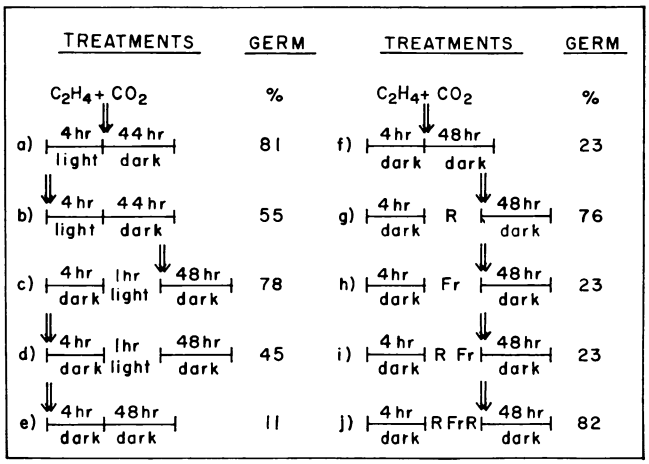

FIG. 6. Effect of different light periods on lettuce seed germination at 35 C. Ethylene (16 μl/l) plus CO_2 (16%) were injected as indicated (see arrows). Light refers to cool white light. R and FR were maintained for 5 min.

cool white light (Fig. 6c). A full 60 min of R resulted in 84% germination (data not shown). The 5-min R treatment was chosen for further study (Fig. 6g). When R was followed immediately by 5 min of FR (Fig. 6i), the germination was reduced to that of the dark control (Fig. 6f); but R given after FR will restore the germination (Fig. 6j). These results are identical to those reported by Borthwick *et al.* (5) for the classical light-sensitive Grand Rapids lettuce seeds. The results also indicate that the phytochrome system is operating at 35 C, and the regulation of germination by C_2H_4 plus CO_2 also depends on the presence of the active form of the phytochrome.

The sensitivity of lettuce seeds used in this study to the gases is altered by changing the duration of dark imbibition (Fig. 7). In curve A seeds were imbibed under air in the dark for 2 to 72 hr. After each selected imbibition time, flasks were purged with air, irradiated with R, and then treated with C_2H_4 plus CO_2. The percentage of germination after 48 hr was plotted. The response to R and gases reached maximum about 16 hr after imbibition. Curve B represents the response to a

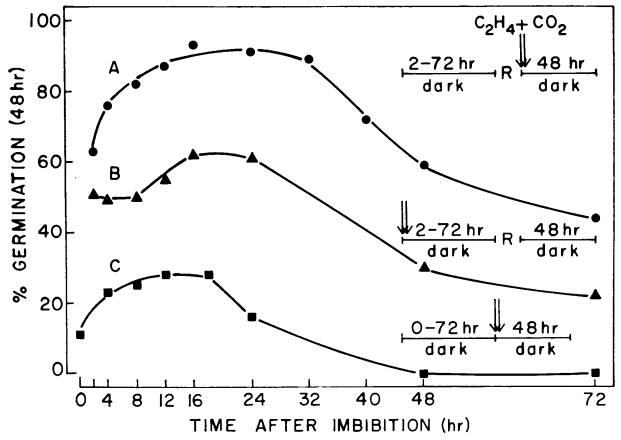

FIG. 7. Variation in germination response of lettuce seeds to C_2H_4 (16 μl/l) plus CO_2 (16%) and R (5 min). Gases were injected as indicated (see arrows).

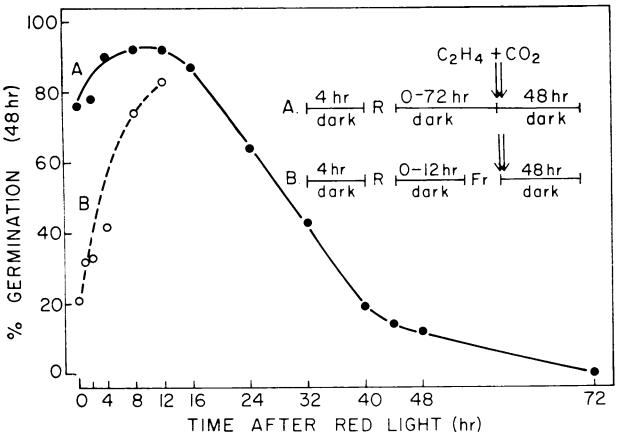

FIG. 8. Postred radiation changes in lettuce seed germination at 35 C (A). Ethylene (16 μl/l) plus CO_2 (16%) were added as indicated (see arrows). The dotted line (B) shows the response to FR given at selected dark intervals after exposure to R.

similar treatment except that gases were added at the start of imbibition. Again there was an inhibitory effect due to the presence of C_2H_4 plus CO_2 at the start of imbibition. Curve C is the dark treatment. It can be seen that the sensitivity to C_2H_4 plus CO_2 passed through a maximum with increasing the time of imbibition. Similarly, adding C_2H_4 plus CO_2 at the start of imbibition reduced the final germination as compared with adding the gases after several hr of imbibition under air. Unless seeds were irradiated with R (Fig. 7, curves A and B), they did not respond to the gases after 48 hr in complete darkness (Fig. 7, curve C).

In order to determine whether one gas or both was responsible for the reduction in germination (Fig. 6, b, and d, and Fig. 7, curve B), seeds were imbibed in the dark for 16 hr in the presence of one gas, irradiated with R for 5 min, and then exposed to the other gas; then the imbibition was continued in the dark for an additional 48 hr. The presence of CO_2 during the first 16 hr before the addition of C_2H_4 resulted in a final reduced percentage of germination which was identical with that when both gases were added at the start of imbibition. Adding C_2H_4 at the start of imbibition, followed later by CO_2 did not reduce the final germination. The presence of both gases just before the light treatment did not reduce the final germination. This might explain the need for longer light exposure to induce maximum germination (Fig. 5).

The use of C_2H_4 plus CO_2 to control germination at 35 C allows a study of the dark changes of phytochrome at this high temperature. Seeds were imbibed under air and in the dark for 4 hr, irradiated with R for 5 min, and kept in the dark from 0 to 72 hr. They were then treated with C_2H_4 plus CO_2 after a selected dark interval, and kept in the dark for an additional 48 hr. The results expressed as percent germination from this experiment are illustrated in Figure 8A. There is an increase in germination for up to 12 hr after R irradiation, although germination begins to decline after that. If the dark period between R and the exposure to gases was increased to 72 hr, the seeds no longer responded to C_2H_4 plus CO_2. On the other hand, if the seeds were reirradiated with R after 24, 48, or 72 hr of dark, and then treated with C_2H_4 plus CO_2, the germination was restored (see Fig. 9, b, d, and f).

Another experiment was carried out to study the persistence of Pfr after R treatment. Seeds were imbibed for 4 hr under air and in the dark, exposed to R for 5 min, then followed by FR at selected intervals from 0 to 12 hr of darkness, and finally treated with C_2H_4 plus CO_2 for 48 hr in the dark. The results are shown in Figure 8B, which suggests that Pfr is converted to another stable product which appears to be FR-insensitive.

It should be mentioned that an atypical germination (4, 18, 30, 33) was observed in some experiments. This occurred only when a dark imbibition period longer than 24 hr (before or after red light treatment) was used before the injection of the gases. The extent of this atypical germination was directly proportional to the length of this dark period.

DISCUSSION

A detailed study of the interaction between C_2H_4 and CO_2 in stimulating the germination of thermodormant lettuce seeds shows that an absence of CO_2 from the atmosphere surrounding the seeds prevents germination (Fig. 2). Increasing the CO_2 concentration in the presence of any C_2H_4 level above 0.8 μl/l stimulates germination to a great extent (Fig. 1). When the gases are added after 48 hr, the results show (Fig. 3B) an absolute requirement for CO_2 when C_2H_4 is used to induce germination in imbibed dormant seeds. These results further confirm those reported previously (1, 27) that CO_2 does not have an effect opposite to C_2H_4 in the germination of lettuce seeds. Recently, others working with the germination of peanuts (21)

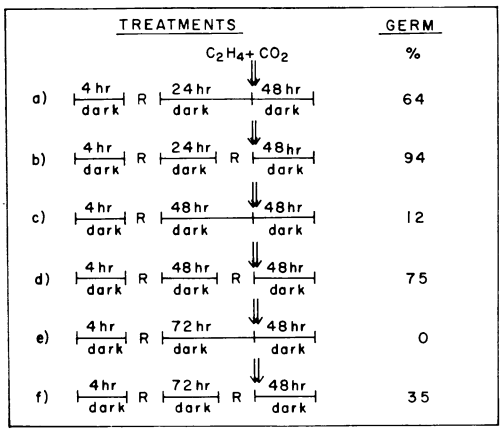

FIG. 9. Effect of additional R (5 min) after selected dark periods on lettuce seed germination at 35 C. Ethylene (16 μl/l) plus CO_2 (16%) were injected as indicated (see arrows).

and Subterranean clover seeds (12) and the growth of rice coleoptile (24) obtained similar responses showing that CO_2 acts synergistically with C_2H_4. On the other hand, Egley and Dale (11) found that CO_2 inhibited the C_2H_4 induced germination of witchweed seeds in a fashion similar to the antagonistic interaction of both gases (10).

The results of Figure 4 suggest that both gases are required for a prolonged period in order to induce maximum germination. This might suggest that a continuous supply of an essential substrate is required for germination at 35 C. The authors feel that more work must be done before any conclusions can be drawn, since adding the gases at the start of imbibition (which was followed during this experiment) causes a lower response than when the gases are added later to previously imbibed seeds.

The enhancement of C_2H_4 action by CO_2 does not seem to be due to a change of the medium's pH. In other systems, such as in the elongation of coleoptile segments (13), a major part of the CO_2 action depends upon its ability to lower the pH of the medium. In seed germination (8, 35), GA needed to be buffered at low pH to have a beneficial effect. In the present study, solutions of low pH did not stimulate germination or replace the requirement for CO_2 (Table I). In the meantime, the addition of CO_2 (in the presence of C_2H_4) to seeds imbibed in acid solution induced as much germination as those imbibed in neutral solution. This indicates that CO_2, rather than bicarbonate, is the active chemical species in stimulating lettuce seed germination at 35 C. A similar conclusion was reached by King and Gould (22) who found that bicarbonate stimulated the germination of *Clostridia* spores at low rather than at high pH. The possibility that lettuce seed endosperm is impermeable to bicarbonate ion (16) is not ruled out. The use of a high concentration of bicarbonate or phosphate buffer reduced the effect of C_2H_4 (Table I), but the presence of C_2H_4 plus CO_2 caused full germination. In experiments not reported here, we studied the response of osmotically inhibited lettuce seeds to C_2H_4 and found that 0.4 M mannitol completely inhibited Great Lakes lettuce seed germination at 25 C in the light for as long as 7 days. Addition of C_2H_4 or C_2H_4 plus CO_2 overcame this inhibition.

Great Lakes lettuce seeds have been generally classified as light-insensitive simply because full germination occurs at 20 to 25 C in light or dark. Many workers induced light sensitivity in these seeds and the seeds of other varieties of lettuce by special treatments (34). After such treatments the so-called light-insensitive seeds became light-requiring and behaved similar to the classically photosensitive Grand Rapids seeds.

By controlling the initiation of germination we were able to demonstrate R-FR reversibility (Fig. 6) and dark changes of the active phytochrome at 35 C (Fig. 8). Phytochrome and the product(s) of the light reaction are shown to be quite stable at 35 C, and the response is identical to those reported for Grand Rapids seeds (5). In the present study, we were able to separate the light reaction from C_2H_4 and CO_2 action. In order for the gases to cause germination there is a requirement for irradiation which gives Pfr or its product(s) or both (Figs. 6 and 8). Other workers reached similar conclusions in studying the interaction between kinetin and light in the germination of lettuce seeds (25) and amaranthin biosynthesis (28) or between GA and light in the germination of lettuce seeds (2). The present work also indicates that Pfr is either converted to another form or is responsible for the formation of some factor (PfrX) (Fig. 8). PfrX could be from a dark conversion of Pfr or it might be an enzyme or other product that is necessary along with C_2H_4 plus CO_2 to cause full germination. The decline in germination by increasing the dark period between R and C_2H_4 plus CO_2 could be due to a turnover of this product or reversion to Pr since reirradiation with red light restores germination (Fig. 9).

Earlier work by Abeles and Lonski (1) showed that C_2H_4 does not act by overcoming dormancy in Grand Rapids lettuce seeds, and that its action is limited to initial steps in the germination process, since treatment of dormant seeds with C_2H_4 had no effect on germination. In our previous report (27), we presented evidence to the contrary and showed that a combination of C_2H_4 with CO_2 will completely overcome lettuce seed dormancy. After discovering that the germination of thermodormant lettuce seeds is under the control of the phytochrome system, we repeated an experiment similar to that reported by Abeles and Lonski (1; see Fig. 2). Figure 9e of the present paper shows that C_2H_4 plus CO_2 will not induce germination when added after 72 hr of dark. This can be explained simply in terms of dark changes of Pfr or its products or both. This is clear in Figure 9f where additional R was given after 72 hr in the dark and before the exposure to C_2H_4 plus CO_2. The low percentage of germination (35%) obtained in this treatment is probably due to a secondary dormancy induced by prolonged incubation in the dark. This type of response has been known for some time (14). The fact that continuous light induced full germination supports this conclusion (27).

Phytochrome changes are known to be temperature-dependent (15). Both inhibition of lettuce seed germination at high temperature and induction of light requirement by incubation at high temperature have been explained in terms of an effect on the phytochrome system (19, 29, 34). Scheibe and Lang (31) concluded that at 37 C Pfr cannot function to promote subsequent germination. The results of this report clearly show that the germination of Great Lakes lettuce seeds at 35 C is under the control of the phytochrome system even though germination does not take place in response to light alone. Furthermore, phytochrome, Pfr, and other products formed as a result of phytochrome changes at 35 C are moderately stable at this temperature. The failure of lettuce seed germination at high temperatures, therefore, is not due to a direct effect on the phytochrome system, but rather to some other blocks. Similar conclusion was reached by other workers (5, 9).

Thornton (33) reported that CO_2 (40–80%) was effective in inducing lettuce seed germination at 35 C in light as well as darkness. The induction of a light requirement in light-insensitive seeds, by incubation at high temperature, has been explained by a thermal inactivation of the active phytochrome. Koller (23) interpreted Thornton's results and suggested that CO_2 exerts a protective effect on the active phytochrome in lettuce seeds, possibly by inactivating the enzyme system which is responsible for the thermal inactivation. Our results do not agree with Koller's interpretation since there is clearly no requirement for C_2H_4 plus CO_2 during the light reaction. Furthermore, we showed that the phytochrome-related changes occur regardless of whether the gases were present or not and C_2H_4 plus CO_2 are able to interact with the products of the light reaction even 24 hr after seeds were exposed to R.

The dark germination of Great Lakes, as well as other varieties of lettuce seeds at 20 to 25 C, has been attributed to the pre-existence of sufficient Pfr in the dry seeds to promote their germination in darkness (17, 26, 34). Boisard *et al.* (3) found a considerable fraction of the phytochrome was present in lettuce seeds in the Pfr form and reported a rapid reversion of Pr to Pfr in the dark. They suggested that this inverse reversion reaction may explain the need for longer FR period to inhibit the dark germination in these seeds. This phenomenon has since been studied in other imbibing seeds (7). Borthwick *et al.* (6), suggested that PfrH, if present, would have absorb-

ance properties noted by Boisard *et al.* and could change in the dark to Pfr. Another possibility suggested by Briggs and Rice (7) is that the inverse reversion could be a hydration phenomenon. Lettuce seeds used in the present study germinate fully in the dark at 25 C. There is a much lower germination in response to C_2H_4 plus CO_2 in darkness at 35 C as compared with those exposed to light. If it is true that Pfr exists in dry lettuce seeds, then why do we not get a maximum germination at 35 C in the dark and in the presence of both gases? Spruit and Mancinelli (32) suggested that the changes in temperature could be responsible for changes in the minimum Pfr level required for the activation of germination or for changes in the rate of inverse dark reversion, or both. It is clear from our results that there are two kinds (possibly closely connected to each other in sequence) of postradiation effects. The first is FR-sensitive Pfr and the second is the FR-insensitive PfrX, which might be the reason for the dark germination of these seeds at 25 C. Other workers (20, 32) have suggested the presence of two pools of phytochrome. The first pool is photostable and the second is produced in the Pr form. Our results could be explained according to this hypothesis if we assume that the first pool is temperature sensitive (stable at 25 C and unstable at 35 C), while the second is fairly stable at 35 C and can be converted by R to Pfr and other products.

Acknowledgments—Seeds were provided through the courtesy of Keystone Seed Company, Hollister, California. The generous donation of a sample of 2444 red Plexiglas from Rohm and Haas is appreciated. The authors wish to thank Drs. B. L. Horst and J. Swader for their helpful discussion and J. Moore for making the illustrations.

LITERATURE CITED

1. Abeles, F. B. and J. Lonski. 1969. Stimulation of lettuce seed germination by ethylene. Plant Physiol. 44: 277–280.
2. Bewley, J. D., M. Negbi, and M. Black. 1968. Immediate phytochrome action in lettuce seeds and its interaction with gibberellins and other germination promoters. Planta 78: 351–357.
3. Boisard, J., C. J. P. Spruit, and P. Rollin. 1968. Phytochrome in seeds and an apparent dark revision of Pr to Pfr. Meded. Landbouwhogeschool Wageningen 68: 1–15.
4. Borthwick, H. A. and W. W. Robbins. 1928. Lettuce seed and its germination. Hilgardia 3: 275–305.
5. Borthwick, H. A., S. B. Hendricks, E. H. Toole, and V. K. Toole. 1954. Action of light on lettuce seed germination. Bot. Gaz. 115: 205–225.
6. Borthwick, H. A., S. B. Hendricks, M. J. Schneider, R. B. Taylorson, and V. K. Toole. 1969. The high-energy light action controlling plant responses and development. Proc. Nat. Acad. Sci. U.S.A. 64: 479–486.
7. Briggs, W. R. and H. V. Rice. 1972. Phytochrome: chemical and physical properties and mechanism of action. Annu. Rev. Plant Physiol. 23: 293–334.
8. Burdett, A. N. 1972. Two effects of prolonged far red light on the response of lettuce seeds to exogenous gibberellin. Plant Physiol. 49: 531–534.
9. Burdett, A. N. 1972. Antagonistic effects of high and low temperature pretreatments on the germination and pregermination ethylene synthesis of lettuce seeds. Plant Physiol. 50: 201–204.
10. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144–152.
11. Egley, G. H. and J. E. Dale. 1970. Ethylene, 2-chloroethylphosphonic acid, and witchweed germination. Weed Sci. 18: 586–589.
12. Esashi, Y. and A. C. Leopold. 1969. Dormancy regulation in subterranean clover seeds by ethylene. Plant Physiol. 44: 1470–1472.
13. Evans, M. L., P. M. Ray, and L. Reinhold. 1971. Induction of coleoptile elongation by carbon dioxide. Plant Physiol. 47: 335–341.
14. Evenari, M. 1965. Light and seed dormancy. *In:* W. Ruhland, ed., Encyclopedia of Plant Physiology, Vol. 15, Part 2. Springer-Verlag, Berlin. pp. 804–847.
15. Furuya, M. 1968. Biochemistry and physiology of phytochrome. *In:* L. Reinhold and Y. Liwschitz, eds., Progress in Phytochemistry, Vol. 1. Wiley, London. pp. 347–405.
16. Haber, A. H. and N. E. Tolbert. 1959. Metabolism of ^{14}C bicarbonate, ^{32}P-phosphate, or ^{35}S-sulfate by lettuce seed during germination. Plant Physiol. 34: 376–380.
17. Hendricks, S. B., E. H. Toole, V. K. Toole, and H. A. Borthwick. 1959. Photocontrol of plant development by the simultaneous excitations of two interconvertible pigments. III. Control of seed germination and axis elongation. Bot. Gaz. 121: 1–8.
18. Ikuma, H. and K. V. Thimann. 1963. Action of kinetin on photosensitive germination of lettuce seed as compared with that of gibberellic acid. Plant Cell Physiol. 4: 113–128.
19. Ikuma, H. and K. V. Thimann. 1964. Analysis of germination processes of lettuce seeds by means of temperature and anaerobiosis. Plant Physiol. 39: 756–767.
20. Kendrick, R. E., C. J. P. Spruit, and B. Frankland. 1969. Phytochrome in seeds of *Amaranthus caudatus*. Planta 88: 293–302.
21. Ketring, D. L. and P. W. Morgan. 1972. Physiology of oil seeds. IV. Role of endogenous ethylene and inhibitory regulators during natural and induced afterripening of dormant Virginia-type peanut seeds. Plant Physiol. 50: 382–387.
22. King, W. L. and G. W. Gould. 1971. Mechanism of stimulation of germination of *Clostridium sporogenes* spores by bicarbonate. *In:* A. N. Barker, G. W. Gould, and J. Wolf, eds., Spore Research 1971. Academic Press, New York. pp. 71–83.
23. Koller, D. 1972. Environmental control of seed germination. *In:* T. T. Kozlowski, ed., Seed Biology, Vol. 2. Academic Press, New York. pp. 1–101.
24. Ku, H. S., H. Suge, L. Rappaport, and H. K. Pratt. 1970. Stimulation of rice coleoptile growth by ethylene. Planta 90: 333–339.
25. Leff, L. 1964. Interaction between kinetin and light on germination of Grand Rapids lettuce seeds. Plant Physiol. 39: 299–303.
26. Mancinelli, A. L. and H. A. Borthwick. 1964. Photocontrol of germination and phytochrome reaction in dark germinating seeds of *Lactuca sativa* L. Ann. Bot. 28: 9–24.
27. Negm, F. B., O. E. Smith, and J. Kumamoto. 1972. Interaction of carbon dioxide and ethylene in overcoming thermodormancy of lettuce seeds. Plant Physiol. 49: 869–872.
28. Nicola, M. G. D., M. Piattelli, V. Castrogiovanni, and C. Molina. 1972. The effect of short-term irradiation on kinetin-induced amaranthin synthesis in *Amaranthus tricolor* seedlings. Phytochemistry 11: 1005–1010.
29. Rollin, P. 1963. Observation sur la différence de deux photo-réactions contrôlant la germination des akènes de *Lactuca sativa* var. "Reine de Mai". Comp. Rend. Acad. Sci. Paris 257: 3642–3645.
30. Scheibe, J. and A. Lang. 1965. Lettuce seed germination: evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect. Plant Physiol. 40: 485–492.
31. Scheibe, J. and A. Lang. 1969. Lettuce seed germination: effects of high temperature and repeated far-red treatment in relation to phytochrome. Photochem. Photobiol. 9: 143–150.
32. Spruit, C. J. P. and A. L. Mancinelli. 1969. Phytochrome in cucumber seeds. Planta 88: 303–310.
33. Thornton, N. C. 1936. Carbon dioxide storage. IX. Germination of lettuce seeds at high temperature in both light and darkness. Contrib. Boyce Thompson Inst. 8: 25–40.
34. Toole, E. H. 1961. The effect of light and other variables on the control of seed germination. Proc. Int. Seed Test. Assoc. 26: 659–673.
35. Toole, V. K. and H. M. Cathey. 1961. Responses to gibberellin of light-requiring seeds of lettuce and *Lepidium virginicum*. Plant Physiol. 36: 663–671.